

APPLICATION  
FOR  
UNITED STATES LETTERS PATENT

TITLE: PLANT TUBBY-LIKE PROTEINS

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# **PLANT TUBBY-LIKE PROTEINS**

## **RELATED APPLICATION**

This application claims priority to U.S. Provisional Application Serial No. 60/441,380, filed on January 21, 2003, the contents of which are incorporated by reference in their entirety.

## **BACKGROUND**

Various environmental factors, e.g., high salinity, pathogens, and chilling, cause stress and adverse effects on growth and productivity of crops. It is therefore desirable to produce transgenic crops that are tolerant to such factors. Genetic engineering can be used to modify proteins that are involved in regulating responses of plants to environmental factors, thereby improving stress-tolerance.

TUBBY proteins, a group of membrane-bound transcription regulators, were first identified from obese mice via positional cloning (Kleyn et al., 1996, Cell 85: 281-290 and Noben-Trauth et al., 1996, Nature 380: 534-538.). Mutations in the TUBBY genes lead to maturity-onset obesity, insulin resistance, retinal degeneration, and neurosensory hearing loss. TUBBY-like proteins (TLPs) were subsequently discovered in other mammals and were found to be activated through G-proteins, which, in higher plants, are involved in the response to environmental factors and hormone regulation (Warpeha et al., 1991, Proc. Natl. Acad. Sci. 88: 8925-8929, and Ueguchi-Tanaka et al., Proc. Natl. Acad. Sci. 97: 11638-11643).

## **SUMMARY**

This invention is based on the discovery of eleven *Arabidopsis* TUBBY-like proteins, designated as AtTLPs 1-11. These proteins regulate the response of *Arabidopsis* to environmental factors. The full-length AtTLPs 1-11 polypeptides (designated as SEQ ID NOs: 1-11), and cDNAs encoding the polypeptides (designated as SEQ ID NOs: 12-22), are shown below:

AtTLP1:

polypeptide:

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1  MSFRSIVRDV RDSIGSLRR SDFKLSSLN KEGKSRGSV QDSHEEQLVV
51  TIQETPWANL PELLRDVIK RLEESVSWP ARRHVVACAS VCRSWRDMCK
101 EIVQSPESG KITFPVSLKQ PGPRDATMQC FIKRDKSNLT YHLYLCLSPA
151 LLVENGKFL SAKRIRRTTY TEYVISMHAD TISRSSNTYI GKIRSNFLGT

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201 KFIYYDTQPA YNSNIARAVQ PVGLSRRFYS KRVSPKVPSG SYKIAQVSYE  
 251 LNVLGTRGPR RMHCAMNSIP ASSLAEGGTV PGQPDII VPR SILDESFESI  
 301 TSSSSRKITY DYSNDFSSAR FSDILGPLSE DQEVVLEEGK ERNSPPLVLK  
 351 NKPPRWHEQL QCWCLNFRGR VTVASVKNFQ LIAANQPQPQ PQPQPQPQPL  
 401 TQPQPSGQTD GPDKI ILQFG KVGKDMFTMD FRYPLSAFQA FAICLSSFDT  
 451 KLACE (SEQ ID NO: 1)

cDNA:

1 ATGTCGTTCC GTAGCATAGT TCGTGATGTG AGAGATAGTA TAGGAAGTCT  
 51 ATCGAGGCGT AGTTTCGACT TTAAGTTAAG CAGCTTGAAC AAAGAAGGTG  
 101 GTAAATCCCG TGGTTCCGTT CAAGATTCTC ATGAGGAACA ACTTGTAGTA  
 151 ACGATTCAAG AAACACCGTG GCGAATCTA CCTCCAGAGT TATTACGTGA  
 201 TGTGATCAAA AGACTTGAAG AGAGTGAAAG TGTGTGGCCT GCTCGTAGAC  
 251 ATGTTGTTGC TTGTGCTTCT GTTTCAGGT CATGGAGAGA TATGTGTAAA  
 301 GAGATTGTTT AAAGTCCGGA GCTCTCAGGC AAAATCACAT TTCCTGTTTC  
 351 GTTGAAACAG CCTGGACCAA GAGATGCAAC AATGCAATGC TTTATCAAAA  
 401 GGGATAAATC TAACTTGACT TATCATTTAT ATCTTTGTCT CAGTCCTGCT  
 451 TTGTTGGTTG AGAATGGAAA GTTCTTCTT TCTGCAAAAC GCATAAGAAG  
 501 AACTACATAC ACCGAGTACG TGATCTCTAT GCACGCCGAC ACCATTTTCGA  
 551 GATCAAGCAA TACCTACATT GGCAAAATCA GGTCTAATTT TCTGGGGACG  
 601 AAGTTTATAA TATACGATAC ACAACCAGCA TACAACAGCA ACATCGCTCG  
 651 AGCGGTCCAA CCGGTAGGTC TTAGCCGAG ATTCTACTCA AAGAGAGTCT  
 701 CTCCCAAAGT ACCTAGTGGG AGCTACAAAA TTGCGCAGGT TTCCTATGAG  
 751 CTAAACGTTT TTGGTACCCG TGGTCCGAGG AGAATGCATT GTGCGATGAA  
 801 CTCAATTCCC GCCTCTTCCC TTGCGGAAGG CGGAACTGTG CCTGGACAGC  
 851 CCGATATCAT TGTCCCGCGC TCTATTCTCG ACGAATCGTT CCGCAGCATT  
 901 ACCTCTTCGT CATCGAGAAA AATCACTTAC GATTACTCGA ATGATTTTAG  
 951 CAGTGCACGG TTTTCCGACA TTCTTGGCCC GTTAAGCGAA GACCAAGAAG  
 1001 TGGTATTAGA AGAAGGGAAA GAGCGGAATT CGCCACCACT TGTGCTTAAG  
 1051 AACAAGCCGC CGAGGTGGCA TGAACAGCTT CAGTGTGGT GTTTAAACTT  
 1101 CAGGGGACGT GTAACAGTCG CATCAGTTAA GAACTTTCAG CTCATTGCAG  
 1151 CAAACCAACC ACAGCCTCAG CCTCAGCCTC AACCGCAACC TCAACCCCTA  
 1201 ACTCAGCCGC AACCGTCTGG TCAGACCGAT GGTCCCGACA AGATCATATT  
 1251 GCAGTTTGGG AAAGTGGGAA AAGACATGTT CACGATGGAT TTCCGGTATC  
 1301 CGCTCTCTGC GTTTCAGGCT TTCGCTATCT GTTTGAGCAG TTTTCGACACA  
 1351 AAACCTTGCTT GCGAA (SEQ ID NO: 12)

AtTLP2:

polypeptide:

1 MSLKSILRDL KEVRDGLGGI SKRSWSKSSH IAPDQTPPL DNIPQSPWAS  
 51 LPPELLHDII WRVEESETAW PARAADVSCA SVCKSWRGIT MEIVRIPEQC  
 101 GKLTFPISLK QPGPRDSPIQ CFIKRNRTA TYILYYGLMP SETENDKLLL  
 151 AARRIRRATC TDFIISLSAK NFRSSSTYV GKLRSGFLGT KFTIYDNQTA  
 201 SSTAQAQPNR RLHPKQAAPK LPTNSSTVGN ITYELNVLRT RGPRRMHCAM  
 251 DSIPLSSVIA EPSVVQIEE EVSSSPSPKG ETITTDKEIP DNSPSLRDQP  
 301 LVLKNKSPRW HEQLQCWCLN FKGRVTVASV KNFQLVAEID ASLDAPPEEH  
 351 ERVILQFGKI GKDIPTMDYR YPLSAFQAFI ICISSEFTKP ACEG (SEQ ID NO: 2)

cDNA:

1 ATGTCTTTGA AAAGCATCCT TCGTGATCTG AAGGAAGTGA GGGATGGACT

51 TGGAGGCATC TCCAAGAGAA GCTGGTCAAA GTCGTCTCAC ATTGCTCCTG  
 101 ATCAAACAAC TCCACCACTG GATAACATAC CACAGAGCCC ATGGGCTTCT  
 151 TTGCCGCTG AGTTGCTTCA TGACATTATC TGGAGGGTTG AAGAGAGTGA  
 201 GACAGCTTGG CCGCTCGAG CTGCCGTTGT CTCTTGCTGCT TCAGTATGTA  
 251 AATCATGGAG AGGAATCACT ATGGAGATTG TGAGGATCCC TGAGCAGTGT  
 301 GGAAGCTCA CTTTTCCAAT CTCATTGAAA CAGCCGGGGC CTCGAGACTC  
 351 TCCAATTCAA TGTTTTATTA AGAGGAACAG AGCAACAGCT ACATACATTC  
 401 TCTATTATGG TTTGATGCCT TCGGAGACTG AGAACGACAA ACTGTTGTTA  
 451 GCAGCAAGAA GGATTAGAAG AGCGACATGC ACAGACTTTA TAATCTCCCT  
 501 ATCTGCAAAG AACTTCTCAC GGAGCAGCAG TACTTATGTT GGCAAATTAA  
 551 GGTCTGGTTT TCTGGGAACC AAGTTCACAA TATATGACAA CCAAAACAGCA  
 601 TCATCCACAG CACAAGCCCA ACCTAACCGA AGACTCCACC CGAAACAAGC  
 651 GGCTCCTAAA CTACCTACGA ATAGCTCTAC CGTAGGAAAC ATAACCTACG  
 701 AGCTCAATGT TCTTCGCACA AGGGGACCTA GAAGAATGCA CTGCCCTATG  
 751 GATTCTATAC CCTCTCTTC TGTATTGCT GAACCGTCAG TAGTTCAAGG  
 801 CATAGAAGAG GAAGTCTCTT CCTCTCCTTC ACCAAAAGGA GAAACCATCA  
 851 CAACAGACAA AGAGATTCTT GATAATTCTC CAAGCTTAAG GGACCAACCG  
 901 CTAGTTCTCA AAAACAAATC CCCAAGATGG CATGAGCAGT TGCAGTGCTG  
 951 GTGCCTCAAC TTCAAGGGAA GAGTGACTGT GGCTTCAGTT AAGAATTTCC  
 1001 AGCTTGTTGC AGAGATTGAC GCTTCTTTGG ATGCCCGGCC TGAAGAACAT  
 1051 GAGAGGTGA TCTTACAGTT TGGCAAAATC GGTAAGGATA TTTTCACCAT  
 1101 GGATTATCGC TACCCTCTAT CTGCTTTTCA AGCCTTTGCT ATATGCATTA  
 1151 GCAGCTTTGA CACCAAACCG GCATGTGAAG GG (SEQ ID NO: 13)

AtTLP3:

polypeptide:

1 MSFKSLIQDM RGEIGSISRK GFDVRFYGR SRSQRVVQDT SVPVDAFKQS  
 51 CWASMPPELL RDVLMRIEQS EDTWPSRKNV VSCAGVCRNW REIVKEIVRV  
 101 PELSSKLTFP ISLKQPGPRG SLVQCYIMRN RSNQTYLYL GLNQAAASND  
 151 GKFLAAKRF RRPTCTDYII SLNCDDVSRG SNYIGKLRS NFLGTFKFTVY  
 201 DAQPTNPGTQ VTRTRSSRL SLKQVSPRIP SGNYPVAHIS YELNVLGSRG  
 251 PRRMQCVMDA IPASAVEPGG TAPTQTEL VH SNLDSFPSFS FFRSKSIRAE  
 301 SLPSGPSSAA QKEGLLV LKN KAPRWHEQLQ CWCLNFNGRV TVASVKNFQL  
 351 VAAPENGPAG PEHENVILQF GKVGKDVFTM DYQYPI SAFQ AFTICLSSFD  
 401 TKIACE (SEQ ID NO: 3)

cDNA:

1 ATGTCCTTCA AGAGTCTCAT TCAGGACATG AGAGGAGAGC TTGGGAGTAT  
 51 ATCCAGAAAG GGATTTCGATG TCAGATTCCG GTATGGTAGA TCCAGGTCTC  
 101 AACGTGTTGT TCAGGATACT TCTGTTCTG TTGATGCTTT CAAGCAGAGC  
 151 TGCTGGGCTA GTATGCCTCC GGAGCTCCTG AGAGATGTTC TTATGAGGAT  
 201 TGAGCAATCC GAAGACACTT GGCCGTCTAG GAAAAATGTT GTTTCTTGGC  
 251 CTGGTGTCTG CAGGAACTGG CGAGAAATCG TCAAAGAGAT CGTCAGAGTT  
 301 CCTGAGCTTT CTAGCAAAC CACTTTTCCT ATCTCCCTCA AACAGCCGGG  
 351 TCCTAGAGGA TCACTGTGTC AATGCTATAT TATGAGAAAC CGCAGCAATC  
 401 AAACCTACTA TCTATACCTC GGGTTAAACC AAGCAGCTTC AAATGATGAT  
 451 GGAAAGTTCC TTCTTGCTGC CAAGAGGTTT CGGAGGCCAA CTGCACTGA  
 501 CTACATCATC TCCTTAAACT GCGATGATGT CTCTCGAGGA AGCAATACCT  
 551 ATATCGGAAA GCTTAGATCT AACTTTCTGG GGACCAAGTT CACTGTCTAT  
 601 GACGCTCAGC CGACGAATCC TGGAACTCAG GTTACCAGAA CCGTTCAAG

651 CAGACTTCTC AGTTTGAAC AAGTGAGCCC GAGAATTCCA TCTGGCAACT  
701 ATCCTGTAGC ACATATCTCA TATGAGCTTA ACGTCTTGGG TTCCAGAGGA  
751 CCGAGGAGGA TGCAGTGTGT CATGGATGCC ATCCCTGCAT CAGCTGTAGA  
801 ACCTGGAGGA ACAGCTCCAA CTCAGACGGA ACTTGTCCAT AGCAATCTTG  
851 ATAGTTTCCC CTCATTCTCC TTCTTCAGGT CGAAATCAAT TCGTGCAGAG  
901 AGTCTCCCTT CTGGTCCATC ATCTGCTGCT CAGAAGGAAG GACTGCTTGT  
951 CCTGAAAAAC AAAGCGCCCA GATGGCACGA ACAGCTCCAG TGCTGGTGCC  
1001 TCAACTTCAA TGGGAGAGTC ACAGTTGCTT CCGTCAAAAA CTITCAGCTG  
1051 GTAGCTGCTC CTGAGAATGG ACCTGCAGGA CCTGAGCACG AAAACGTGAT  
1101 TCTCCAGTTT GGAAAAGTCG GAAAAGATGT GTTCACAATG GATTATCAGT  
1151 ACCCTATCTC TGCCTTCCAG GCCTTCACCA TTTGCCTCAG CAGTTTCGAC  
1201 ACCAAGATAG CATGTGAA (SEQ ID NO:14)

AtTLP4:

polypeptide:

1 MPPELLRDVL MRIERSEDW PSRKNVSCV GVCKNWRQIF KEIVNVPEVS  
51 SKFTFPISLK QPGPGGSLVQ CYVKRNRNQ TFYLYLGGEA KIFCQSEPSD  
101 IYLVPSYSRE THCVMDAISA SAVKPGGTAT TQTELDNFVS FRSPSQKEG  
151 VLVLSKSVPR LEEQSWCLDF NGSPENEPEN ENDIFQFAKV GNLHKLFSLY  
201 EAEWIPLVRT SVFAVIARVC RDKKHTPSYE LKLALYFAKN SAILKKFVLR  
251 GYTREEDLLA LPVAN (SEQ ID NO:4)

cDNA:

1 ATGCCTCCTG AGCTTCTGAG AGATGTTCTG ATGAGGATAG AGCGATCCGA  
51 AGACACTTGG CCTTCTAGGA AGAATGTTGT TTCTTGTGTA GGTGTGTGTA  
101 AGAACTGGCG ACAAATATTC AAAGAGATCG TTAACGTTCC TGAGGTTTCT  
151 AGCAAATTCA CTTTTCCAAT CTCCTTGAAA CAGCCTGGTC CAGGAGGATC  
201 ACTTGTTCAT TGCTATGTTA AGAGAAACCG TAGCAATCAA ACTTCTATC  
251 TATACCTTGG AGGTGAAGCA AAAATATTTT GTCAGTCTGA ACCAAGTGAT  
301 ATTTATCTCG TTCTTACAG TTACAGAGAG ACGCATTGCG TCATGGATGC  
351 CATCTCTGCA TCAGCAGTAA AACCTGGAGG AACAGCTACA ACTCAGACAG  
401 AACTCGATAA TTTCGTGTCA TTCAGGTCTC CTCTGGTCA AAAGGAAGGA  
451 GTGCTTGTTT TTAAGAGCAA AGTGCCTAGA TTGGAAGAAC AGAGCTGGTG  
501 TCTCGACTTC AATGGCTCTC CTGAGAACGA ACCTGAGAAT GAAAACGACA  
551 TTTTCCAGTT TCGGAAAGTC GGAAACTTGC ACAAACCTTT CAGTTTATAT  
601 GAGGCTGAAT GGATTCTCTT CGTTCGCACC TCAGTGTGTTG CTGTCATTGC  
651 TCGAGTTTGT AGAGATAAAA AGCATACACC ATCGTATGAA TTGAAACTTG  
701 CATTGTACTT TGCAAAAAAC TCTGCAATCC TCAAGAAATT CGTTCTCCGC  
751 GGTTACACTC GAGAAGAAGA TTTACTCGCA TTGCCCGTGG CTAAC (SEQ ID NO:15)

AtTLP5:

polypeptide:

1 MSFLSIVRDV RDTVGSFSRR SFDVRSNGT THQRSKSHGV EAHIEDLIVI  
51 KNTRWANLPA ALLRDVMKKL DESESTWPAR KQVVACAGVC KTWRLMCKDI  
101 VKSPEFSGLK TFPVSLKQPG PRDGIIQCYI KRDKSNMTYH LYLSPAIL  
151 VESGKFLLSA KRSRRATYTE YVISMDADNI SRSSSTYIGK LKSNFLGTFK  
201 IVYDTAPAYN SSQILSPNR SRSFNSKKVS PKVPSGSYNI AQVTYELNLL  
251 GTRGPRRMC IMHSIPSLAL EPGGTVPSPQ EFLQSLDES FRSIGSSKIV  
301 NHSGDFTRPK EEEGKVRPLV LKTKPPRWLQ PLRCWCLNFK GRVTVASVKN  
351 FQLMSAATVQ PGSGSDGGAL ATRPSLSPQQ PEQSNHDKII LHFQKVGKDM

401 FTMDYRYPLS AFQAF AISLS TFDTKLACE (SEQ ID NO: 5)

cDNA:

1 ATGTCGTTTC TGAGTATTGT TCGTGATGTT AGAGATACTG TAGGAAGCTT  
 51 TTCGAGACGT AGTTTCGACG TGAGAGTATC TAATGGGACG ACTCATCAGA  
 5 GGAGTAAATC TCACGGTGTG GAGGCACATA TTGAAGATCT TATTGTAATC  
 101 AAGAACACTC GTTGGGCTAA TTTACCGGCT GCGCTATTAC GAGATGTGAT  
 151 GAAAAAGTTG GATGAAAGCG AGAGTACTTG GCCTGCACGT AAACAAGTCG  
 201 TTGCTTGTGC TGGTGTCTGC AAGACATGGA GACTAATGTG CAAAGATATT  
 251 GTGAAAAGTC CTGAGTTCTC AGGCAAACTC ACATTTCCAG TTTCGTTGAA  
 10 301 ACAGCCCGGG CCTAGGGATG GAATCATACA ATGTTATATC AAAAGAGACA  
 351 AGTCTAACAT GACTTACCAC CTTTACCTTT CTCTTAGTCC TGCCATACTT  
 401 GTTGAAGTG GGAAGTTTCT TCTCTCGCA AAGCGCTCAC GGAGAGCTAC  
 451 ATACACAGAG TATGTAATAT CAATGGATGC AGACAACATT TCAAGATCAA  
 501 GCAGCACTTA CATTGGCAAA CTGAAGTCTA ACTTTCTAGG GACAAAATTT  
 15 601 ATAGTATATG ATACGGCTCC TCGGTACAAC AGTAGCCAGA TATTGTCCCC  
 651 ACCAAACCGG AGTCGTAGTT TCAACTCCAA GAAAGTGTCT CCCAAAGTCC  
 701 CTTCTGGAAG TTACAACATT GCTCAAGTTA CATAAGAGCT GAACTTGCTT  
 751 GGAACCCGTG GACCTCGTAG AATGAAGTGC ATTATGCACT CTATCCCCTC  
 801 CTTAGCTCTA GAACCCGAG GACTGTCCC TAGCCAACCT GAGTTTCTAC  
 20 851 AACGTTCCCT TGATGAATCT TTCCGCAGCA TCGGTTCCCT AAAGATAGTC  
 901 AACCACTCGG GAGATTTCAC CCGACCGAAA GAGGAAGAAG GAAAGGTGCG  
 951 ACCTTTGGTA CTGAAAATA AACCGCCAAG GTGGCTCCAA CCGTTGCGAT  
 1001 GTTGGTGCCT TAACCTCAAA GGGAGAGTGA CTGTAGCTTC TGTCAGAAGC  
 1051 TTCCAGTTGA TGTCCGCTGC AACGGTTCAG CCCGGTAGTG GTAGTGATGG  
 25 1101 TGGAGCATTG GCTACGAGGC CATCGTTATC ACCACAGCAG CCAGAGCAAT  
 1151 CAAACCATGA TAAGATAATA CTACACTTTG GGAAAGTGGG TAAGGATATG  
 1201 TTCATATGG ACTATCGTTA TCCTCTCTCT GCCTTTCAAG CGTTTGCCAT  
 1251 TTCCCTGAGC ACCTTTGATA CTAAATTGGC ATGTGAA (SEQ ID NO: 16)

AtTLP6:

polypeptide:

1 MSLKNIVKNK YKAIGRRGRS HIAPEGSSVS SSLSTNEGLN QSIWDLPEE  
 51 LLLDIIQRIE SEQSLWPGRR DVVACASVCK SWREMTKEVV KPPELSGLIT  
 101 FPISLRQPGP RDAPIQCFIK RERATGIYRL YLGLSPALSG DSKLLLSAK  
 35 151 RVRRTGAEF VVSLSGNDFS RSSSNYIGKL RSNFLGTFKFT VYENQPPPFN  
 201 RKLPPSMQVS PWVSSSSSSY NIASILYELN VLRTRGPRRM QCIMHSIPIS  
 251 AIQEGGKIQS PTEFTNQKK KKKPLMDFCS GNLGGESVIK EPLILKNKSP  
 301 RWHEQLQCWC LNFKGRVTVA SVKNFQLVAA AAEAGKNMNI PEEEQDRVIL  
 351 QFGKIGKDIF TMDYRYPISA FQAF AICLSS FDTKPVCE (SEQ ID NO: 6)

cDNA:

1 ATGTCATTGA AGAACATAGT GAAGAACAAA TACAAAGCTA TTGGTAGAAG  
 51 AGGGAGGTCA CACATTGCAC CAGAAGGATC ATCTGTGTCT TCCTCTTTAT  
 101 CAACTAATGA AGGTTTAAAC CAGAGTATTT GGGTTGATTT GCCTCCAGAG  
 151 TTA CTCTTG ATATAATCCA AAGGATTGAG TCTGAACAGA GTTTATGGCC  
 201 GGGGAGGAGA GATGTTGTG CTGTGCTTC GGTGTTGTAAG TCATGGAGGG  
 251 AGATGACTAA AGAAGTTGTT AAAGTTCCTG AGCTCTCTGG TTTGATCACG  
 301 TTTCCGATTT CTTTAAGACA GCCTGGACCT AGAGATGCTC CAATTCAATG  
 351 CTTTATTAAA CGTGAAAGAG CTACGGGGAT ATACCGTCTC TATCTTGGTT  
 401 TAAGCCCTGC TCTTCCGGT GACAAGAGTA AGTTGTTGTT ATCTGCAAAG

5 451 AGAGTCAGGA GAGCGACGGG TCGGAGTTT GTTGTATCGT TATCGGGGAA  
 501 TGACTTCTCG AGAAGTAGTA GTAATTACAT AGGAAAACTG AGATCAAATT  
 551 TCCTGGGAAC GAAGTTCACA GTCTACGAAA ACCAACCTCC TCCGTTTAAC  
 601 CGAAAGCTCC CACCATCGAT GCAAGTGTCT CCATGGGTAT CGTCGTCATC  
 651 TAGTAGTTAC AACATAGCTT CAATCTTGTA TGAGCTGAAT GTTCTGAGAA  
 701 CCAGAGGTCC AAGAAGAATG CAATGTATAA TGCACAGTAT CCGGATTTCA  
 751 GCGATTCAAG AAGGCGGCAA AATCCAGTCG CCAACGGAGT TCACAAACCA  
 801 AGGAAAGAAG AAGAAGAAGC CGCTGATGGA TTTCTGCTCA GGGAACTGG  
 851 GAGGAGAATC CGTTATAAAA GAACCATTAA TTCTGAAAA CAAGTCGCCG  
 10 901 AGATGGCAGC AACAGCTTCA GTGCTGGTGT CTAAACTTCA AAGTCGAGT  
 951 CACAGTCGCC TCGGTGAAAA ACTTCCAGCT AGTGGCAGCT GCTGCAGAA  
 1001 CAGGGAAGAA CATGAACATA CCAGAAGAGG AACAAGATAG AGTGATATTA  
 1051 CAGTTTGGGA AGATAGGCAA AGACATTTTC ACAATGGATT ATCGTTACCC  
 1101 GATCTCTGCA TTCCAAGCTT TTGCTATTTG TTAAAGCAGC TTCGACACGA  
 15 1151 AGCCAGTCTG CGAA (SEQ ID NO:17)

AtTLP7:

polypeptide:

20 1 MPLSRSLLSR RISNSFRFHQ GETTTAPESE SIPPPSNMAG SSSWSAMLPE  
 51 LLGEIIRVE ETEDRWQRR DVVTCACVSK KWREITHDFA RSSLSNGKIT  
 101 FPSCLKLPGP RDFSNOCLIK RNKKTSTFYI YLALTPSFTD KGKFLAARR  
 151 FRTGAYTEYI ISLDADDFSQ GSNAYVGKLR SDFLGTNFTV YDSQPPHNGA  
 201 KPSNGKASRR FASKQISPOV PAGNFEVGHV SYKFNLLKSR GPRRMVSTLR  
 25 251 CPSPSPSSSS AGLSSDQKPC DVTKIMKKPN KDGSSLTILK NKAPRWHEHL  
 301 QCWCLNFHGR VTVASVKNFQ LVATVDQSQP SGKGDEETVL LQFGKVGDDT  
 351 FTMDYRQPLS AFQAFACLIT SFGTKLACE (SEQ ID NO:7)

cDNA:

30 1 ATGCCTTTGT CACGGTCCTT CCTTTCGCGG AGGATCTCGA ACTCTTTTAG  
 51 GTTTCATCAG GGAGAGACAA CGACGGCACC GGAATCCGAA TCGATTCTCT  
 101 CGCCGTCGAA TATGGCCGGT TCTTCGTCAT GGTCGGCGAT GCTCCCTGAA  
 151 TTATTAGGCG AGATCATTCG TCGCGTGGAG GAGACTGAGG ACCGTTGGCC  
 201 TCAACGTCGT GATGTAGTTA CTTCGCGCTG CGTTTCTAAG AAATGGAGAG  
 251 AAATCACTCA CGATTTCTCT AGATCCTCTC TTAACCTCTG CAAAATTACT  
 301 TTCCCTTCTT GCCTCAAATT GCCAGGTCCT AGAGACTTTT CTAATCAGTG  
 35 351 CTTGATAAAG AGGAACAAGA AGACATCAAC GTTTTACTTG TATCTTGCTC  
 401 TAACACCATC ATTCATGAT AAGGGAAAGT TTCTTCTGGC GGC GCGGAGG  
 451 TTTAGGACCG GTGCTTACAC TGAGTACATC ATATCACTTG ATGCTGATGA  
 501 TTTCTCTCAA GGAAGTAATG CCTACGTCGG AAAATTAAGA TCAGATTTTC  
 551 TTGGGACCAA CTTTACAGTA TACGATAGCC AACCACCACA CAACGGAGCA  
 40 601 AAACCTTCAA ATGGCAAAGC CAGTCGCAGA TTTCATCAA AGCAGATAAG  
 651 CCCTCAAGTT CCAGCAGGCA ACTTTGAAGT CGTCATGTT TCTTATAAAT  
 701 TCAACCTTTT GAAATCAAGA GGTCCAAGAA GAATGGTAAG CACACTCCGA  
 751 TGCCCATCAC CATCACCTTC ATCATCATCC GCTGGACTCT CGTCTGACCA  
 801 AAAGCCATGT GATGTAACCA AGATAATGAA AAAACCCAAC AAGGATGGTT  
 45 851 CCAGCTTGAC AATACTAAAG AACAAAGCTC CTAGATGGCA CGAGCACTTG  
 901 CAGTGCTGGT GTCTGAACTT CCATGGACGA GTTACTGTTG CTTCGGTCAA  
 951 GAACCTTCAG CTGGTTGCGA CCGTTGACCA AAGTCAACCG AGCGGTAAAG

1001 GCGATGAAGA AACAGTTCTT CTACAGTTTG GTAAAGTGGG AGATGACACT  
 1051 TTCACTATGG ATTATAGACA GCCTCTCTCT GCATTTTCAGG CTTTTGCTAT  
 1101 CTGTCTCACA AGTTTCGGCA CTAAACTTGC CTGCGAG (SEQ ID NO:18)

5 AtTLP8:

polypeptide:

1 MAGSRKVNDL LEENKGNVDT ITGSLSTQKG EDKENVSPEK VSTSIVETRL  
 51 DRALKSQSMK GNSGFPTTEVT NFKSFSTGGR TALKQSSLQA CMQKNSEVDK  
 101 SSFGMKTWTS VDSEHSSSLK VWEFSDSEAA PASSWSTLPN RALLCKTLPL  
 151 DVGRCTCLIV KEQSPEGLSH GSVYSLYTHE GRGRKDRKLA VAYHSRRNGK  
 201 SIFRVAQNVK GLLCSSDESY VGSMTANLLG SKYYIWDKGV RVGSVGMVK  
 251 PLLSVVIFTP TITWTGSYR RMRTLLPKQQ PMQKNNNKQV QQASKLPLDW  
 301 LENKEKIQKL CSRIPHYNKI SKQHELDPRD RGRGLRIQS SVKNFQLTLT  
 351 ETPRQTILQM GRVDKARYVI DFRYPFSGYQ AFCICLASID SKLCCTV (SEQ ID NO:8)

15 cDNA:

1 ATGGCTGGTT CGAGAAAAGT GAATGATTTG TTGGAGGAAA ATAAGGGAAA  
 51 TGTGGACACA ATTACAGGT CTTTATCCAC TCAAAGGGA GAGGATAAGG  
 101 AGAATGTGTC GCCGGAGAAA GTCTCTACCT CTGTGGAAAC TCGGAAACTA  
 151 GATCGAGCTT TGAAGTCTCA ATCGATGAAG GGTAAGTCTG GGTTCCTAAC  
 201 GGAAGTTACA AATTTCAAAT CTTTCTCAAC TGGTGGTCGA ACAGCTCTGA  
 251 AGCAGTCATC ACTGCAAGCG TGTATGCAGA AGAACAGTGA GGTGATAAG  
 301 AGTAGTTTCG GAATGAAAAC TTGGACTAGT GTTGATTGAG AGCATTCAAG  
 351 TTCGTTGAAA GTGTGGGAGT TTTCGGATTC TGAAGCTGCC CCTGCTTCCT  
 401 CTTGGTCTAC TTGCCCCAAC AGGGCTTTGT TGTGCAAGAC ACTACCTTTG  
 451 GATGTGGGAA GATGCACTTG TCTGATTGTG AAAGAACAAT CACCTGAAGG  
 501 CTTGAGCCAC GGATCTGTAT ATTCACITTA TACACATGAA GGTGCGGGGC  
 551 GTAAAGACCG GAAGTTAGCA GTTGCTTACC ATAGCCGACG TAATGGGAAA  
 601 TCTATATTTA GGGTGGCACA GAATGTTAAG GGATTGCTGT GCAGTTCCGA  
 651 TGAAAGTTAT GTCGGTTCCA TGACGGCTAA TCTCTTGGGT TCCAAGTACT  
 701 ACATATGGGA CAAGGGAGTT CGAGTTGGTT CTGTAGGTAA AATGGTGAAG  
 751 CCGCTTCTTT CGGTTGTAAT ATTCACACCC ACCATAACAA CTTGGACAGG  
 801 GAGCTACAGA AGAATGAGAA CTTTGCTACC AAAGCAGCAG CCAATGCAGA  
 851 AAAACAACAA TAAGCAGGTT CAACAAGCTA GTAAACTACC GCTTGATTGG  
 901 CTTGAGAATA AGGAAAAAAT TCAGAAGCTA TGCTCAAGGA TACCACATTA  
 951 CAACAAAATC TCCAAGCAGC ATGAGTTAGA CTTAGAGAC AGAGGAAGAA  
 1001 CAGGACTGAG AATACAGAGC TCGGTGAAGA ACTTTCAGCT AACACTCACG  
 1051 GAGACTCCAA GGCAGACAAT TCTTCAAATG GGGAGAGTTG ACAAAGCAAG  
 1101 ATATGTAATC GACTTCAGGT ATCCATTCTC AGGCTACCAA GCATTCTGCA  
 1151 TTTGCTTGGC TTCTATTGAT TCCAAGCTTT GTTGACTGT T (SEQ ID NO:19)

40 AtTLP9:

polypeptide:

1 MTFRSLQEM RSRPHRVVHA AASTANSSDP FSWSELPEEL LREILIRVET  
 51 VDGGDWPSRR NVVACAGVCR SWRILTKEIV AVPEFSSKLT FPISLKQSGP  
 101 RDSLVQCFIK RNRNTQSYHL YLGLTTS LTD NGKFLAASK LKRATCTDYI  
 151 ISLRSDDISK RSNAYLGRMR SNFLGTFKTV FDGSQTGA AK MQKSRSSNFI  
 201 KVSPPVQGS YPIAHISYEL NVLGSRGPRR MRCIMDTIPM SIVESRGVVA  
 251 STSISSFSSR SSPVFRSHSK PLRSNSASCS DSGNNLGDPP LVLSNKA PRW  
 301 HEQLRCWCLN FHGRVTVASV KNFQLVAVSD CEAGQTSERI ILQFGKVGKD

cDNA:

351 MFTMDYGYPI SAFQAFACL SSFETRIACE (SEQ ID NO:9)

1 ATGACGTTCC GAAGTTTACT CCAGGAAATG CCGTCTAGGC CACACCGTGT  
 51 AGTTCACGCC GCCGCCTCAA CCGCTAATAG TTCAGACCCT TTCAGCTGGT  
 5 CGAGAGCTCCC GGAGGAGCTG CTTAGAGAAA TCCTGATTAG GGTGAGACT  
 101 GTTGACGGCG GCGATTGGCC GTCGCGGCGA AACGTGGTGG CTTGTGCCGG  
 151 CGTTTGTCTG AGCTGGAGGA TTCTACACAA GGAGATTGTA GCTGTTCCTG  
 201 AATTCTCCTC TAAATTGACT TTCCCTATCT CCCTCAAGCA GTCTGGTCCA  
 251 AGAGATTCTC TAGTTCAATG CTTTATAAAA CGTAATCGAA ATACTCAATC  
 301 GTATCATCTC TATCTCGGAT TAACTACCTC TTTGACGGAT AACCGGAAGT  
 351 TTCTTCTTGC TGCTTCTAAG CTGAAGCGCG CAACTTGAC TGAATACATC  
 401 ATCTCTTTGC GTTCAGACGA TATCTCAAAG AGAAGCAACG CGTATCTTGG  
 451 GAGAATGAGA TCGAACTTCC TTGGAACAAA ATTCACGGTC TTTGATGGTA  
 501 GTCAGACCGG AGCAGCGAAG ATGCAGAAGA GCCGCTCTTC TAATTTCATC  
 551 AAAGTTTCAC CTAGAGTTCC TCAGGGAAGT TACCCCATCG CTCACATTTT  
 601 ATACGAGTTA AACGTCTTAG GCTCTCGGGG ACCGAGAAGA ATGCGTTGCA  
 651 TCATGGATAC AATACCTATG AGCATCGTGG AGTCGCGAGG AGTAGTAGCT  
 701 TCAACATCCA TAAGCTCTTT TTCCAGTCGG TCATCACCAG TCTTTAGGTC  
 751 TCACTCAAAA CCATTGCGCA GTAATAGTGC ATCATGTAGC GACTCAGGCA  
 801 ACAACCTGGG AGATCCACCA TTGGTGCTGA GCAACAAAGC TCCACGGTGG  
 851 CATGAGCAGT TACGTTGCTG GTGCTTAAAT TTCCATGGTC GAGTCACAGT  
 901 GGCTTCGGTT AAGAACTTTC AGCTTGTGGC AGTTAGTGAC TGTGAAGCAG  
 951 GGCAGACATC TGAGAGGATC ATACTCCAGT TTGGGAAAGT TGGGAAGGAC  
 1001 ATGTTTACCA TGGATTATGG ATATCCGATT TCTGCGTTTC AAGCGTTTGC  
 1051 TATCTGCCTG AGCAGTTTGT AAACCAGAAT TGCTGTGAA (SEQ ID NO:20)

AtTLP10:

polypeptide:

1 MSFRGIVQDL RDGFGSLRR SDFRLSSLH KGKAQGSSFR EYSSSRDLLS  
 30 51 PVIVQTSRWA NLPPELLFDV IKRLEESN WPARKHVAC ASVCRSWRAM  
 101 CQEIVLGPFI CGKLTFPVS LKQGPDRAMI QCFIKRDKSK LTFHLFLCLS  
 151 PALLVENGKF LLSAKRTRRT TRTEYIISMD ADNISRSSNS YLGKLSNLF  
 201 GTKFLVYDTQ PPPNTSSAL ITDRTSRSRF HSRRVSPKVP SGSYNIAQIT  
 251 YELNVLGTRG PRMHCI MNS IPISLEPGG SVPNQPEKL PAPYSLDDSF  
 301 RSNISFSKSS FDRSLDFSS SRFSEMGISC DDNEEASFR PLILKNQPR  
 351 WHEQLQCWCL NFRGRVTAS VKNFQLVAAR QPQPQGTGAA APTSAPAHF  
 401 EQDKVILQFG KVGKDMFTMD YRYPLSAFQA FAICLSSFD T KLACE (SEQ ID NO:10)

cDNA:

1 ATGTCGTTTC GAGGCATTGT TCAAGATTG AGAGATGGGT TTGGGAGCTT  
 40 51 GTCAAGGAGG AGTTTCGATT TTAGGCTCTC GAGTCTTCAT AAAGGAAAG  
 101 CTCAGGGTTC TTCGTTCCGT GAGTATTCGT CATCCCGTGA TCTCTGTGCG  
 151 CCTGTGATAG TTCAGACAAG TAGATGGGCT AATCTTCCTC CAGAGTTACT  
 201 CTTTGATGTG ATCAAAAGAT TAGAGGAAAG TGAGAGTAAT TGGCCTGCAA  
 251 GAAAACATGT TGTGGCTGTG GCTTCGGTTT GTCGGTCTTG GAGAGCTATG  
 301 TGCCAAGAGA TTGTTTTGGG GCCTGAAATC TGTGGGAAAC TCACTTTCCC  
 351 TGTTTCCCTC AAACAGCCAG GGCTCGTGA TGCAATGATT CAGTGTTCAT  
 401 TCAAAAGGGA TAAATCAAAG CTAACATTTT ACCTTTTCT TGTGTTAAGT  
 451 CCCGCTCTAT TAGTGGAGAA TGGGAAATTT CTTCTTTCAG CTAAGAAGAC  
 501 TCGTAGAACT ACTCGAACCG AGTACATTAT CTCCATGGAT GCTGATAACA

551 TCTCAAGATC CAGCAACTCT TACCTCGGAA AGCTCAGATC AAACTTCCTT  
601 GGGACAAAGT TCTTGGTGTA CGACACGCAA CCACCACCAA ACACATCTTC  
651 GAGCGCACTT ATCACTGATC GAACAAGCCG AAGCAGGTTT CACTCCAGAC  
701 GAGTTTCTCC TAAAGTACCA TCCGGAAGCT ACAACATTGC TCAAATCACC  
751 TATGAGCTCA ACGTGTGGG CACACGCGG CCACGACGAA TGCACTGCAT  
801 CATGAAGTCC ATCCCAATTT CATCGCTCGA ACCAGGCGGT TCAGTCCCTA  
851 ACCAACCCGA GAAACTCGTC CCTGCACCAT ACTCTCTCGA CGACTCATTC  
901 CGCAGTAACA TCTCCTTCTC CAAATCATCA TTTGACCACC GCTCCCTCGA  
951 TTTCAGCAGT TCTAGATTCT CCGAAATGGG AATATCCTGC GACGACAACG  
1001 AAGAAGAAGC GAGTTTCAGA CCGTTGATTC TAAAGAACAA GCAGCCAAGG  
1051 TGGCAGCAGC AGTTGCAATG CTGGTGTGTTG AATTTCCGCG GACGTGTGAC  
1101 AGTTGCATCG GTTAAGAATT TCCAGCTTGT AGCAGCAAGA CAGCCGCAGC  
1151 CTCAAGGGAC AGGTGCAGCA GCAGACCAA CAAGTGCACC TGCTCACCCT  
1201 GAGCAAGACA AGGTGATTCT CCAGTTTGGT AAAGTAGGGA AAGATATGTT  
1251 CACAATGGAC TATAGGTATC CATTATCGGC GTTTCAGGCG TTTGCGATAT  
1301 GCTTAAGCAG CTTTGACACC AAGCTTGCTT GTGAA (SEQ ID NO:21)

AtTLP11:

polypeptide:

1 MRSRPHRVVH DLAAAAADS TSVSSQDYRW SEIPEELLRE ILIRVEAADG  
51 GGWPSRRSVV ACAGVCRGWR LLMNETVVVP EISSKLTFFI SLKQPGPRDS  
101 LVQCFIKRNR ITQSYHLYLG LTNSLTDDGK FLLAACKLKH TTCTDYIISL  
151 RSDDMSRRSQ AYVGKVRSNF LGTKFTVFDG NLLPSTGA AK LRKRSYNPA  
201 KVS AKVPLGS YPVAHITYEL NVLGSRGPRK MQCLMDTIPT STMEPQGVAS  
251 EPSEFLLGT RSTLSRSQSK PLRSSSSHLK ETPLVLSNKT PRWHEQLRCW  
301 CLNFHGRVTV ASVKNFQLVA AGASCGSGTG MSPERQSERI ILQFGKVGKD  
351 MFTMDYGYPI SAFQAFACL SSFETRIACE (SEQ ID NO:11)

cDNA:

1 ATGCGTTTGA GACCGCATCG TGTGGTCCAC GACCTTGCCG CGCGCCGAGC  
51 TGCCGATTCC ACTTCTGTGT CATCGCAAGA TTATCGCTGG TCAGAGATTG  
101 CTGAAGAGCT TCTTAGGGAG ATTCTGATTC GTGTTGAAGC GGCGGACGGT  
151 GGCGGATGGC CGTCACGACG CAGCGTGGTG GCTTGTGCCG GCGTTTGTCTG  
201 TGGCTGGCGG CTACTTATGA ACGAAACCGT CGTTGTCCCT GAGATCTCTT  
251 CTAAGTTGAC TTTCCCCATC TCTCTCAAGC AGCCTGGTCC AAGGGATTCA  
301 CTGGTTCAAT GCTTTATCAA ACGTAATCGA ATTACGCAAT CATATCATCT  
351 CTATCTCGGA TTAACCAACT CTTTAACGGA TGATGGGAAG TTTTTCCTTG  
401 CTGCGTGTA GGTGAAGCAC ACAACTTGTA CGGATTACAT TATCTCTTTA  
451 CGTTCTGATG ATATGTCGAG AAGAAGCCAA GCTTATGTTG GCAAAGTGAG  
501 ATCGAACTTC CTAGGAACGA AATCACTGT CTTTGATGGA AATCTGCTGC  
551 CTTCAACGGG AGCCGCAAAG TTGAGAAAGA GCCGATCTTA TAATCCCGCA  
601 AAAGTTTCAG CAAAAGTTCC TCTTGGAAGT TATCCTGTG CTATATCAC  
651 ATATGAGCTG AATGTCTTAG GATCCCGGGG ACCAAGAAAG ATGCAATGTC  
701 TTATGGACAC AATACCTACA AGCACAATGG AGCCTCAAGG AGTAGCTTCA  
751 GAACCATCAG AGTTTCCCTT ACTCGGTA CTGTTCAACCT TATCCAGGTC  
801 TCAGTCAAAA CCATTACGCA GTAGCTCAAG CCACCTGAAA GAAACACCAT  
851 TAGTGCTGAG CAACAAGACA CACGGTGGC ACGAGCAGCT ACGTGTCTGG  
901 TGCTTGAATT TCCATGGCCG TGTACAGTA GCGTCAGTGA AGAACTTTCA  
951 GCTCGTGGCA GCAGGAGCTA GCTGTGGCAG TGGCACGGGA ATGTACCGG  
1001 AGAGGCAGAG CGAGCGGATT ATATTGCAGT TTGGGAAAGT CGGGAAAGAT

1051 ATGTTACGA TGGATTATGG ATACCGATC TCAGCTTTCC AGGCTTTTGC  
 1101 CATTTGCTTG AGCAGCTTTG AGACTAGAAT CGCTTGTGAA (SEQ ID NO:22)

Accordingly, one aspect of the invention features an isolated polypeptide containing an amino acid sequence at least 70% (i.e., any number between 70% and 100%, inclusive) identical to one of SEQ ID NOs: 1-11. When expressed in a plant cell, e.g., an *Arabidopsis* cell, the polypeptide regulates the transcription of genes, in response to environmental stimuli. The polypeptide of the invention can be used to identify DNA elements, such as promoters, enhances, or silencers, which it binds to. Such DNA elements mediate the response of plants to various environmental factors. The polypeptide of the invention can also be used for producing anti-AtTLP antibodies (either monoclonal or polyclonal). These antibodies in turn are useful for detecting the presence and distribution of AtTLP proteins in tissues and in cellular compartments. For example, such antibodies can be used to verify the expression of TLP proteins in a transgenic plant.

An isolated polypeptide refers to a polypeptide substantially free from naturally associated molecules, i.e., it is at least 75% (i.e., any number between 75% and 100%, inclusive) pure by dry weight. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

The percent identity of two amino acid sequences is determined using the algorithm of Karlin and Altschul ((1990) Proc. Natl. Acad. Sci. USA 87, 2264-2268), modified as in Karlin and Altschul ((1993) Proc. Natl. Acad. Sci. USA 90, 5873-5877). Such an algorithm is incorporated into the XBLAST programs of Altschul, et al. ((1990) J. Mol. Biol. 215, 403-410). BLAST protein searches are performed with the XBLAST program, score = 50, wordlength = 3. Where gaps exist between two sequences, Gapped BLAST is utilized as described in Altschul, et al. ((1997) Nucleic Acids Res. 25, 3389-3402). When employing BLAST and Gapped BLAST programs, one can conveniently use the default parameters (e.g., XBLAST). See [ncbi.nlm.nih.gov](http://ncbi.nlm.nih.gov).

The invention further features (1) an isolated nucleic acid encoding a polypeptide of the invention and (2) an isolated nucleic acid that, under a high stringent condition, hybridizes to a probe containing a sequence selected from the group consisting of SEQ ID NOs: 12-22, or a complement of any selected sequence. Such a nucleic acid is at least 15 (e.g., at least 30, 50, 100, 200, 500, or 1000) nucleotides in length. By hybridization under a high stringent condition

is meant hybridization at 65°C, 0.5 X SSC, followed by washing at 45°C, 0.1 X SSC. The nucleic acids of the invention can be used to determine whether an AtTLP mRNA is expressed in a tissue or cell. The nucleic acids can be used as primers in PCR-based detection methods, or as labeled probes in nucleic acid blots (e.g., Northern blots).

5 An isolated nucleic acid refers to a nucleic acid the structure of which is not identical to that of any naturally occurring nucleic acid or to that of any fragment of a naturally occurring genomic nucleic acid. The term therefore covers, for example, (a) a DNA which has the sequence of part of a naturally occurring genomic DNA molecule but is not flanked by both of the coding sequences that flank that part of the molecule in the genome of the organism in which  
10 it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a  
15 fusion protein.

The invention also features a vector and a host cell containing a nucleic acid of the invention. The host cell can be an *E. coli.*, a yeast, an insect, a plant (e.g., *Arabidopsis*), or a mammalian cell. The vector and host cell can be used for producing a polypeptide of the invention. For this purpose, one can culture the host cell in a medium under conditions  
20 permitting expression of the polypeptide, and isolate the polypeptide.

The just-described vector and host cell can also be used for generating a transformed plant cell or a transgenic plant containing a recombinant nucleic acid that encodes a heterologous polypeptide of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. One can generate such a transformed plant cell by introducing into a plant cell a recombinant nucleic acid encoding such  
25 a heterologous polypeptide and expressing the polypeptide in the cell. To generate a transgenic plant, one can (1) introduce into a plant cell a recombinant nucleic acid encoding one just-described heterologous polypeptide; (2) expressing the polypeptide in the cell, and (3) cultivating the cell to generate a plant. The transformed plant cell or transgenic plant is more sensitive to environmental factors, such as high salinity, pathogens, and chilling, and therefore can be used as  
30 a sensor to detect and monitor small changes in environment, such as soil and air.

Also within the scope of this invention are a homozygous transformed plant cell (e.g., an

Arabidopsis cell) and a transgenic plant (e. g. Arabidopsis) that lack a polypeptide containing a sequence of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. The transformed plant cell or transgenic plant, compared with the wild type cell or plant, has a higher (by at least 30%, e.g., 50%, 90%, 100%, 200%) tolerance to salt, chilling, pathogens, oxidative stress, or water-deficit due to absence of or lowered level of the polypeptide. In addition, the invention features method of making the transformed plant cell and the transgenic plant. Both methods include introducing into a plant cell a nucleic acid (e.g., a T-DNA, an anti-sense RNA, and an iRNA) that decreases the expression of a gene encoding a polypeptide of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. The method for making the plant further includes cultivating the plant cell to generate a plant.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

### DETAILED DESCRIPTION

This invention is based on an unexpected discovery that (1) overexpression of AtTLPs in *Arabidopsis* increases sensitivity of the plant to various environmental factors, such as salt, chilling, oxidative stress, or water-deficit; and (2) lack of expression of AtTLPs increases tolerance of the plant to several environmental factors.

Accordingly, in one aspect, the invention features a transformed plant cell containing a recombinant nucleic acid that encodes a heterologous AtTLP. The AtTLP proteins useful for this invention include, *Arabidopsis* AtTLPs 1-11 and TLPs of other species. The plant cell can be a dicot plant cell (e.g., a tomato cell, a brassica cell, or a potato cell) or a monocot plant cell (e.g. a rice cell, a wheat cell, or a barley cell).

A transformed plant cell of the invention can be produced by introducing into a plant cell a recombinant nucleic acid that encodes a heterologous AtTLP protein and expressing the protein in the cell. Techniques for transforming a wide variety of plant cells are well known in the art and can be found in technical and scientific literature. See, for example, Weising et al., 1988, Ann. Rev. Genet. 22:421-477. To express a heterologous AtTLP gene in a plant cell, the gene can be combined with transcriptional and translational initiation regulatory sequences that direct the transcription of the gene and translation of the encoded protein in the plant cell.

For overexpression, a constitutive plant promoter may be employed. A constitutive

promoter is active under most environmental conditions and states of cell differentiation.

Examples of constitutive promoters include the cauliflower mosaic virus (CaMV) 35S transcription initiation region, the 1'- or 2'-promoter derived from T-DNA of *Agrobacterium tumefaciens*, the ACT11 and Cat3 promoters from *Arabidopsis* (Huang et al., 1996, *Plant Mol. Biol.* 33:125-139 and Zhong et al., 1996, *Mol. Gen. Genet.* 251:196-203), the stearyl-acyl carrier protein desaturase gene promoter from *Brassica napus* (Solomon et al., 1994, *Plant Physiol.* 104:1167-1176), and the Gpc1 and Gpc2 promoters from maize (Martinez et al., 1989, *J. Mol. Biol.* 208:551-565 and Manjunath et al., 1997, *Plant Mol. Biol.* 33:97-112).

Alternatively, a tissue-specific promoter or an inducible promoter may be employed to direct expression of the AtTLP gene in a specific cell type or under more precise environmental or developmental control. Examples of environmental conditions that may affect transcription by inducible promoters include anaerobicity, elevation of temperature, presence of light, spray with chemicals or hormones, or infection by a pathogen. Examples of a tissue-specific promoter or an inducible promoter include the root-specific ANR1 promoter (Zhang and Forde, 1998, *Science* 279:407) and the photosynthetic organ-specific RBCS promoter (Khouli et al., 1997, *Gene* 197:343).

For proper polypeptide expression, a polyadenylation region at the 3'-end of the coding region should be included. The polyadenylation region can be derived from the same gene, from a variety of other genes, or from T-DNA.

A marker gene can also be included to confer a selectable phenotype on plant cells. For example, the marker gene may encode a protein that confers biocide resistance, antibiotic resistance (e.g., resistance to kanamycin, G418, bleomycin, hygromycin), or herbicide resistance (e.g., resistance to chlorosulfuron or Basta).

A recombinant nucleic acid that encodes a heterologous AtTLP protein may be introduced into the genome of a desired plant host cell by a variety of conventional techniques. For example, the recombinant nucleic acid may be introduced directly into the genomic DNA of a plant cell using techniques such as polyethylene glycol precipitation, electroporation, microinjection, or ballistic methods (e.g., DNA particle bombardment). See, e.g., Paszkowski et al., 1984, *EMBO J.* 3:2717-2722, Fromm et al., 1985, *Proc. Natl. Acad. Sci. USA* 82:5824, and Klein et al., 1987, *Nature* 327:70-73. Alternatively, the recombinant nucleic acid may be combined with suitable T-DNA flanking regions and introduced into a conventional

Agrobacterium tumefaciens host vector. The virulence functions of the Agrobacterium tumefaciens host direct the insertion of the AtTLP gene and adjacent marker into the plant cell DNA when the cell is infected by the bacteria. Agrobacterium tumefaciens-mediated transformation techniques, including disarming and use of binary vectors, are well known in the art. See, e.g., Horsch et al., 1984, Science 233:496-498; Fraley et al., 1983, Proc. Natl. Acad. Sci. USA 80:4803; and Gene Transfer to Plants, Potrykus, ed., Springer-Verlag, Berlin, 1995.

The presence and copy number of a heterologous AtTLP gene in a transgenic plant can be determined using standard methods, e.g., Southern blotting. Expression of the heterologous AtTLP gene in a transgenic plant can be confirmed by detecting and quantifying the heterologous AtTLP mRNA or protein in the transgenic plant.

The transformed plant cells thus obtained can then be cultured to regenerate a whole plant. Regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide or herbicide marker that has been introduced together with a heat shock factor gene. Plant regeneration from cultured protoplasts is described in Evans et al., Protoplasts Isolation and Culture, Handbook of Plant Cell Culture, pp. 124-176, MacMillan Publishing Company, New York, 1983; and Binding, Regeneration of Plants, Plant Protoplasts, pp. 21-73, CRC Press, Boca Raton, 1985. Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee et al., 1987, Ann. Rev. Plant Phys. 38:467-486. Once the heterologous AtTLP gene has been confirmed to be stably incorporated in the genome of a transgenic plant, it can be introduced into other plants by sexual crossing. Depending upon the species to be crossed, one or more standard breeding techniques can be used to generate the whole plant.

In another aspect, the invention feature a homozygous transformed plant cell that lack one or more of AtTLPs 1-11. Absence of the AtTLP(s) enhances tolerance of the cell to various environmental factors, e.g., high salinity. Such a transformed cell can be made by introducing into a plant cell a nucleic acid that lowers the expression of a gene encoding a polypeptide of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. The nucleic acid, e.g., T-DNA, antisense RNA, or iRNA, can be introduced into the cell using one of the standard transforming techniques described above. Stable transformants can be selected using the marker genes and selection methods also described above. A whole plant can then be regenerated from the transformed plant cells. It can be further crossed using conventional breeding techniques to generate

homozygous plant.

The specific example below is to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. All publications cited herein are hereby incorporated by reference in their entirety.

#### Identification of the AtTLP Family

A tubby consensus sequence (Pfam PF01167, Kleyn et al., 1996, Cell 85: 281-290 and Noben-Trauth et al., 1996, Nature 380: 534-538) was used to search the *Arabidopsis thaliana* expressed sequence tag (EST) database and the completed Arabidopsis genome sequence (The Institute of Genome Research, TIGR) with multiple BLAST algorithms to locate all the sequences sharing significant similarities with the tubby domain (P-value < 0.0085). The search results revealed eleven TUBBY-like protein genes, termed AtTLP1 to AtTLP11, in the Arabidopsis genome. For each of the 11 genes, the corresponding BAC locus (The Arabidopsis Information Resource), Tentative Consensus (TC) group, AGI gene code, cDNA GenBank accession number, and predicted protein length (No. of amino acid) are summarized in Table 1 below.

**Table 1. AtTLP family members**

Gene Name	BAC Locus	TC group	AGI Gene Code	cDNA GenBank Accession No.	Predicted Protein Length
AtTLP1	F22K20.1	TC95487	At1g76900	AF487267	455
AtTLP2	T30D6.21	TC86308	At2g18280	AY045773	394
AtTLP3	F17A22.29	TC86633	At2g47900	AY045774	406
AtTLP4	F8K4.13	-	At1g61940	-	265
AtTLP5	T10P12.9	TC102456	At1g43640	AY046921	429
AtTLP6	F8G22.1	TC90700	At1g47270	AF487268	388
AtTLP7	F12M16.22	TC88599	At1g53320	AY092403	379
AtTLP8	T24D18.17	-	At1g16070	AF487269	397
AtTLP9	F24P17.15	TC102624	At3g06380	AF487270	380
AtTLP10	F4F7.13.	TC101291	At1g25280	AF487271	445
AtTLP11	T1A4.60	-	At5g18680	AY046922	380

Gene-specific 5' and 3' primers were designed based on the sequence of the predicted open reading frame (ORF) and the corresponding EST in the database. The primer pairs used are listed below:

5           AtTLP1-5' (5'-ATGTCGTTCCGTAGCATAGTTCGT-3')  
             AtTLP1-3' (5'-TTATTCGCAAGCAAGTTTTGTGTCG-3')  
             AtTLP2-5' (5'-ATGTCTTTGAAAAGCATCCTTCGTGATC-3')  
             AtTLP2-3' (5'-TTACCCTTCACATGCCGGTTTGGTGTCA-3')  
             AtTLP3-5' (5'-ATGTCCTTCAAGAGTCTCATTTCAG-3')  
 10          AtTLP3-3' (5'-TCATTCACATGCTATCTTGGTGTC-3')  
             AtTLP5-5' (5'-ATGTCGTTTCTGAGTATTGTTCG-3')  
             AtTLP5-3' (5'-TTATTCACATGCCAATTTAGTAT-3')  
             AtTLP6-5' (5'-ATGTCATTGAAGAACATAGTGAA-3')  
             AtTLP6-3' (5'-TCATTCGCAGACTGGCTTCGTGT-3')  
 15          AtTLP7-5' (5'-ATGCCTTTGTACGGTCCCTC-3')  
             AtTLP7-3' (5'-TCACTCGCAGGCAAGTTTAGTG-3')  
             AtTLP8-5' (5'-ATGGCTGGTTCGAGAAAAGTGAA-3')  
             AtTLP8-3' (5'-TCAAACAGTACAACAAAGCTTGG-3')  
             AtTLP9-5' (5'-ATGACGTTCCGAAGTTTACTCCA-3')  
 20          AtTLP9-3' (5'-TTATTCACAGGCAATTCTGGTTT-3')  
             AtTLP10-5' (5'-ATGTCGTTTCGAGGCATTGTTCA-3')  
             AtTLP10-3' (5'-CTATTCACAAGCAAGCTTGGTGT-3')  
             AtTLP11-5' (5'-ATGTCGTTTCTGAGTATTGTTCG-3')  
             AtTLP11-3' (5'-TTATTCACATGCCAATTTAGTAT-3')

25

RT-PCR was then performed using total RNA from 2-week-old Arabidopsis seedlings. The total RNA was isolated using the TRIZOL reagent (Invitrogen) according to the manufacturer's direction. PolyA<sup>+</sup>-mRNA was isolated using oligo (dT)-coated magnetic beads and the PolyAtract system (Promega, Madison, WI). First strand cDNA was synthesized from 0.5 µg PolyA<sup>+</sup>-mRNA using SuperScript II RNase H Reverse Transcriptase (Invitrogen) according to the protocol of the supplier.

30

The above-described gene-specific primer pairs were used for amplifying cDNA of each AtTLP gene from first-strand cDNA. PCR conditions were as follows: 3 min at 94°C; 25 cycles of 1 min denaturation at 94°C/ 1 min annealing at 55°C/1 min 30 s extension at 72°C. The PCR products were purified using the QIAquick PCR purification kit (Qiagen) and subcloned into a T-easy vector (Promega). Each of these clones was verified by sequencing. Ten AtTLP cDNAs, AtTLPs 1-3 and AtTLPs 5-11, were successfully amplified.

It was found that, except for AtTLPs 2 and 11, the amino acid sequences deduced from the cDNA sequences of AtTLPs 1, 3, and 5-10 are identical to the predicted ORFs in the database. The analysis of the AtTLP2 cDNA sequence indicated that its intron3 was located between 708-781 bp whereas the predicted splicing sites for this intron located were 663 and 766 bp. The analysis of AtTLP11 cDNA sequence showed that intron2 and intron4 were located at 669-803 bp and 1334-1575 bp, respectively, whereas the computer predicted intron2 was at 621-803 bp and there was no predicted intron4. All cDNA sequences obtained from this study were submitted to GenBank.

#### Sequence Analysis of AtTLP Proteins

The search for all known motifs in the deduced AtTLP amino acid sequences was conducted by MOTIF SCANNING (Pagni et al., 2001, Nucleic Acids Res 29: 148-151). Multiple sequence alignment was performed using ClustalW (Thompson et al., 1994). This analysis revealed that each AtTLP gene, except AtTLPs 4 and 8, had a well-conserved tubby domain at its C-terminus. Unlike animal TLPs, which have highly diverse N-terminal sequences, each AtTLP, except AtTLP8, had a conserved F-box (51-57 residues) containing domain (Pfam PF00646).

Pair-wise comparisons among the AtTLP proteins revealed that their tubby domains shared 30% to 80% similarities. Further analyzing the tubby domain revealed two PROSITE signature patterns: TUB1 (Prosite Accession No. PS01200) and TUB2 motif (Prosite Accession No. PS01201). The TUB1 and TUB2 motifs were located at the C-terminal of each AtTLP protein and contain 14 and 16 amino acid residues, respectively. These two TUB motifs are highly conserved among TLPs from various organisms. Though AtTLP4 and 8 do not have obvious TUB1 and TUB2 motifs, their C-terminal tubby domains are recognizable by MOTIF SCANNING (N-score >15) (Pagni et al., 1993, Nucleic Acids Res 29: 148-151).

An obvious feature of AtTLPs is the tubby domain. In the tubby domain of a mouse TUBBY protein, three positively-charged amino acid residues, R332, R363 and K330, were thought to be crucial for PI (4,5) P<sub>2</sub> binding (Santagata et al., 2001, Science 292: 2041-2050). A sequence alignment of AtTLP tubby domains with the mouse TUBBY domain revealed a putative PI (4,5) P<sub>2</sub> binding domain in each AtTLP, except AtTLPs 4 and 8. This suggests that AtTLPs 1-3, 4-7, and 8-11 may bind to PI (4,5) P<sub>2</sub>. It is known that the mouse TUBBY protein is a bipartite transcription regulator. Its tubby domain possesses double-stranded DNA binding activity, and its N-terminal segment seems to modulate transcription (Boggon et al., 1999, Science 286: 2119-2125). In plants, the N-terminal region of TLPs is quite different from that in mammal TLPs as AtTLP9-GAL4 DNA binding domain fusion protein failed to activate transcription from a GAL4 promoter in a heterologous system.

#### Location and Gene Structure Comparison of the AtTLP Gene Family

Chromosome localizations of each AtTLP genes were determined using Map View (www.arabidopsis.org/servlets/mapper) (Huala et al., 2001, Nucleic Acids Res 29: 102-105). It was found that the genes were not evenly distributed on chromosomes I, II, III, and V. Seven genes (AtTLPs 1, 4, 5, 6, 7, 8, and 10) were located on chromosome I, and two genes (AtTLPs 2 and 3) were located on chromosomes II. The other two, AtTLPs 9 and 11, were located on chromosomes II, III respectively. Although most of the AtTLP genes were located on chromosome I, no local tandem repeats or gene clusters were identified.

By comparing the sequences of the RT-PCR products and the Arabidopsis genome, the corrected exon-intron organizations of the AtTLP genes (except for AtTLP4) were determined. It was found that exon 1 contained the sequences encoding each protein's N-terminal leading sequence, the F-box, and a nine-residue spacer between the F-box and tubby domain. This result indicated that the genes might have arisen from the same ancestral gene. The sequence encoding the C-terminal tubby domain was found to distribute in exons interrupted by 2 or 3 introns. On the basis of the exon and intron composition, the AtTLP genes were classified into three groups. Each gene of the first group (AtTLPs 1, 2, 5, 6, 7, and 10) contains three introns. Each of the second group, AtTLPs 3, 9 and 11, contains an additional intron in the region encoding the C-terminal part of the tubby domain. The third and the most distinct group (AtTLPs 4 and 8) contain 5 and 8 introns, respectively.

Expression of AtTLP Genes

A coupled RT-PCR based assay was conducted to determine the expression pattern of AtTLP genes. Total RNA was isolated from roots, main and lateral stems, rosette leaves, flower clusters, and green siliques of 42-days-old soil-grown Arabidopsis. For each gene, a pair of gene-specific primers was chosen, and PCR amplifications were carried out using 15 ng of first strand cDNA synthesized as described above. Primers of ubiquitin gene, UBQ10, (5'-ATTTCTCAAAATCTTAAAACTT-3' and 5'-TGATAGTTTTCC CAGTCAAC-3') were used to amplify ubiquitin, which served as an internal loading standard (Norris et al., 1993, Plant Mol. Biol. 21: 895-906).

The results showed that AtTLPs 1, 2, 3, 6, 7, 9, 10 and 11 were expressed in all organs tested, with slight variations in mRNA accumulation. In contrast, AtTLPs 5 and 8 were primarily expressed in the root, flower, and silique. The organ-specific expressions of AtTLPs 5 and 8 indicate their specific roles in particular organs.

Although the expression of AtTLP1, 2, 3, 6, 7, 9, 10 and 11 is present in all tissues tested, the possibility that these genes are expressed with cell type specificity could not be excluded. It is possible that differential expression of these AtTLP genes could only be observed when internal developmental programming was altered or specific environmental stimuli were applied to the plants. To test this hypothesis, the public Arabidopsis Functional Genomics Consortium (AFGC) microarray expression database (the Stanford Microarray Database, genome-www5.stanford.edu/MicroArray/SMD/) (Wu et al., 2001, Plant Physiol Biochem 39: 917-926) was searched. Twofold expression was used as the difference cutoff. Based on the search, the expression profiles of DNA fragments corresponding to AtTLP2, 7, 9 and 10 were summarized in Table 2 below.

**Table 3 Microarray analysis of *AtTLP* genes expression**

Experiment	Channel 1 Description	Channel 2 Description	Ch2/Ch1 Normalized (Mean) <sup>b, c</sup>			
			<i>AtTLP2</i>	<i>AtTLP7</i>	<i>AtTLP9</i>	<i>AtTLP10</i>
<b>Hormone Effect</b>						
Auxin Response	msg seedlings, untreated	msg seedlings, 10 uM IAA for 30 min	0.32	2.21		
Auxin Induction	Mock-treated Columbia roots	NAA-treated Columbia roots		0.46		2.22
Cytokinin response	Control	15 min cytokinin treatment	2.19			
Absciscic acid Insensitive 1 <i>edr1</i> Mutant	Wild type control	<i>Absciscic acid insensitive 1</i> mutant	0.49	2.6	0.35	
Downstream genes of <i>KN1</i>	Control	Overexpression of <i>KN1-GR</i> in Columbia-0 background	0.43	2.78		4.73
<b>Stress</b>						
Effects of Elevated atmospheric CO <sub>2</sub>	Columbia leaves 360ppm CO <sub>2</sub>	Columbia leaves 1000ppm CO <sub>2</sub>	0.33			
Genes involved in chilling tolerance	Cold treated Columbia wild type tissue [K+]=120uM	Cold treated <i>cls8</i> mutant tissue [K+]=2mM	0.22		0.15	
Genes involved in potassium nutrition				0.34		0.2
Cadmium	Control	10uM cadmium treated plant			2.72	
<b>Light Signaling</b>						
Circadian rhythm time = 12,0 Phototropic stimulation	Time = 0 hrs Seedlings grown in the dark and exposed to 1hr blue light Wild type	Time = 12 hrs <i>nph4-2</i> seedlings grown in the dark and exposed to 1 hr blue light <i>cia-2</i> (mutant)	0.37 2.08			
Protein import into chloroplasts: CIA-2						3.46
Identification of genes in chlorophyll starvation	WT leaves after exposure to 230uE for 2 days	<i>cchl1</i> leaves after exposure to 230 uE for 2 days				2.02
<b>Stress</b>						
Effects of Elevated Atmospheric CO <sub>2</sub>	Columbia leaves 360ppm CO <sub>2</sub>	Columbia leaves 1000ppm CO <sub>2</sub>	0.33			
Genes involved In chilling tolerance	Cold treated Columbia wild type tissue [K+]=120uM	Cold treated <i>cls8</i> mutant tissue [K+]=2mM	0.22		0.15	
Genes involved in potassium nutrition				0.34		0.2
Cadmium	Control	10uM cadmium treated plant			2.72	

<sup>a</sup> These data are obtained from [http://afgc.stanford.edu/afgc\\_html/site2.htm](http://afgc.stanford.edu/afgc_html/site2.htm)

<sup>b</sup> All data are corresponding with fluorescence intensities greater than 500 in both channels and ch2/ch1 normalized ratio  $\geq 2.0$  or  $\leq 0.5$

<sup>c</sup> When searching dbEST with blastn, we find *Arabidopsis* EST corresponding to fragments of four *AtTLPs* represented on microarray data generated by AFGC. *AtTLP2* is corresponding to the EST clone 289B10T7 and 173K22T7. *AtTLP7* *AtTLP9* and *AtTLP10* are corresponding to the EST clone 173G1T7, 201E19T7 and F3E6T7, respectively.

The resulted show that factors like hormone fluctuation and environmental stimuli modulate the expression of the four *AtTLP* genes. As shown in Table 2, the four *AtTLP* genes

had different responses to treatments of various hormones. AtTLP2 gene expression instantaneously increased more than twofold with cytokinin treatment but decreased to one-third after being treated with IAA. This suggests that Cytokinin and auxin play antagonistic roles in regulating AtTLP2 gene expression.

Another cytokinin-related experiment was aimed at identifying downstream genes of KN1. KN1-like protein is a homeobox transcription factor. Its overexpression upregulates cytokinin production and leads to delayed senescence (Vollbrecht et al., 1991, Nature 350: 241-243). The expression of AtTLP7 and 10 is upregulated in KN1 overexpression transgenic plant while AtTLP2 is downregulated.

The different responses of AtTLPs 7, 2, and 10 to ABA treatment is also worth noticing. In abscisic acid insensitive 1 mutant (Pei et al., 1997, Plant Cell 9: 409-423), the expression of AtTLP2 and AtTLP10 decreases by two to threefold, but AtTLP7 expression increases over twofold. Interestingly, AtTLP2 and AtTLP7 behaved oppositely to auxin treatment and in abscisic acid insensitive 1 mutant and KN1 overexpression transgenic plant. These two AtTLPs therefore may function antagonistically in regulating phytohormone-signaling pathways.

The expression level of AtTLP2 rose in the *edr1* (enhanced disease resistance I) mutant leaves. The EDR1 gene encodes a putative MAP kinase similar to CTR1, a negative regulator of ethylene response in Arabidopsis (Frye et al., 2001, Proc. Nat. Acad. Sci. 98: 373-378). The *edr1* mutation of Arabidopsis also confers resistance to powdery mildew disease (Frye and Innes, 1998, Plant Cell 10: 947-956). Thus, the regulation of AtTLP2 gene expression may be associated with SA-inducible and ethylene defense mechanism.

Environmental stresses also impose influences on the expression of AtTLP genes. For example, similar to the cold treatment on *cls8* mutant, elevated CO<sub>2</sub> level inhibited the expression of AtTLP2. K<sup>+</sup> deficiency augmented the expression of AtTLPs 7 and 10 by threefold and fivefold, respectively. Heavy metal cadmium treatment stimulated the expression of AtTLP9.

In conclusion, the expression data of these four AtTLP genes indicate their involvement in phytohormone and environmental stress signaling.

AtTLP9 Interacts with ASK1 Protein

Homology searches in the public databases reveal that TLPs were also present in multiple plant species, including *Lemna paucicostata*, *Oryza sativa*, *Cicer arietinum*, maize, and *Arabidopsis*. Unlike animal TLPs having highly diverse N-terminal sequences, plant TLPs had conserved F-box-containing domain. Sequence alignment of the F-box cores from AtTLP, TIR, UFO, COI1 and the human F-box protein Skp2 revealed conserved islands separated by regions with weak homology. Many of the conserved residues correspond with those known to be important for Skp association (Schulman et al., 2000, Nature 408: 381-386 and Zheng et al., 2002, Nature 416: 703-709).

The F-box domain, first found in cyclin-F, interacts with the protein SKP1, which interacts with the Cdc53 (Cullin) proteins, to form a so-called SCF complex. The F-box is involved in recruiting specific proteins (e.g., transcription activators or repressors) and targeting them for ubiquitin-mediated proteolysis by 26S proteasome. Analysis of the *Arabidopsis* genome revealed that *Arabidopsis* had 21 Skp1-like, or ASK, protein, which exhibited different expression patterns. Among them, ASK1 is involved in vegetative growth and reproductive development (Zhao et al., 2003, Plant Physiology 133: 203-217).

To test whether AtTLP could interact with ASK1, AtTLP9 was examined by the yeast two-hybrid analysis. Yeast two-hybrid vectors, pAD-GAL4-2.1 and pBD-GAL4 Cam (Stratagene, La Jolla, CA), were used for C-terminal GAL4 AD and BD fusion constructions, respectively. A 1.1-kb Sall-PstI fragment containing the entire coding region of AtTLP9 was cloned into the Sall-PstI site of the pBD-GAL4 Cam vector. A 480-bp EcoRI-PstI fragment containing the entire coding region of ASK1 (At1g75950) was cloned into the EcoRI-PstI site of the pAD-GAL4-2.1 vector. The yeast strain YRG-2 [MATa ura3-52 his3-200 ade2-101 lys2-801 trp1-901 leu2-3,112 gal4-542 gal80-538 LYS2::GAL1UAS-GAL1TATA-HIS3 URA3:: $(\text{GAL43} \times 17\text{mer})$ -CYC1TATA-lacZ] was co-transformed with the two vectors. The Y2H analysis was performed according to the manufacturer's recommendations (Stratagene). The result suggested that AtTLP9 physically interacts with ASK1 to form SCF complex and acts as a factor for substrate recognition in the ubiquitin-mediated proteolysis.

### Attlp9 Null Mutants and Overexpression Lines

ATLP9 was analyzed to investigate for it in vivo functions. Both loss-of-function and overexpression approaches were taken to address its biological roles.

To identify attlp9 T-DNA insertion mutant, AtTLP9 (At3g06380) was used to search the T-DNA Express database at <http://signal.salk.edu/cgi-bin/tdnaexpress>. Two attlp9 T-DNA insertion mutants (ABRC seed stock SALK\_016678 and 051138) were identified and designated as attlp9-1 and attlp9-2. T<sub>3</sub> seeds of attlp9-1 and attlp9-2 were obtained from the Arabidopsis Biological Resource Center (Ohio State University, Columbus). The position of the T-DNA within the AtTLP9 gene was re-confirmed by sequencing a PCR-amplified fragment amplified by primer pairs corresponding to the T-DNA left borders and the AtTLP9 gene specific primer. The following primer pairs were used for attlp9-1 and attlp9-2 specific amplification,

attlp9-1: N1, 5' -ATGACGTTCCGAAGTTTACTC- 3';

LBa1, 5' -TGGTTCACGTAGTGGGCCATC- 3';

attlp9-2: C1, 5' -TTATTCACAGGCAATTCTGGT- 3'; and

LBa1, 5' -TGGTTCACGTAGTGGGCCATC- 3'.

It was found that Attlp9-1 had a T-DNA insertion in the coding sequence at codon 705, whereas attlp9-2 had an insertion in the 5' distal region of this gene. The T-DNA insertion site of attlp9-1 was identical to that originally described in the T-DNA Express database. However, the T-DNA insertion site of attlp9-2 was in the promoter region instead of exon1 as predicted in the database (the latter is supported by a potential full length cDNA corresponding to At3g06380 generated in RIKEN, accession number BT004092).

Southern blot was conducted with the nptII marker gene to determine the T-DNA insertion number in attlp9-1 and 9-2 knockout mutants. It was found that one and three T-DNA insertion events in the T<sub>4</sub> attlp9-1 and attlp9-2 T-DNA insertion mutants, respectively.

The T-DNAs in attlp9-1 and attlp9-2 carried a gene leading to resistance to kanamycin. Homozygous analyses of attlp9-1 and attlp9-2 plants were carried out by kanamycin selection and PCR based method. RT-PCR analyses of T<sub>4</sub> homozygous of attlp9-1 and attlp9-2 plants indicated that attlp9-1 was a null allele, whereas attlp9-2 was somewhat leaky. For the phenotype investigation, attlp9-1 and attlp9-2 T<sub>4</sub> homozygous lines were used for detailed analysis.

Transgenic plants with overexpressing AtTLP9 were generated. An XbaI-SmaI fragment of AtTLP9 was inserted into an XbaI-SmaI site of the pBI121 Ti-vector (Clontech) to generate a 35S:: AtTLP9 sense construct. The XbaI-SmaI fragment contained the entire AtTLP9 coding region and was under the control of the 35S promoter of cauliflower mosaic virus. The constructs were introduced into *Agrobacterium* strain LBA4404 by electroporation and transformed into wild-type plants by the floral dip method (Clough et al., 1998, *Plant J* 16: 735-743). 38 independent transgenic lines ( $T_0$  generation) were obtained. Among them, seven independent homozygous lines from the  $T_3$  sense transgenic plants were analyzed for the AtTLP9 expression. Each of these lines contained a single copy of the transgene. Two independent transgenic lines (S13-2 and S16-1) showed dramatic increases in the AtTLP9 transcript levels and were further analyzed. A number of control transgenic lines were generated by transforming with *Agrobacterium* with PBI121 vector alone.

The wild type *Arabidopsis thaliana* ecotype Columbia-0 (Col-0) and mutant *abi4-1* (obtained from Dr Wan-Hsing Cheng, Institute of Botany, Academia Sinica Taipei) were used. The phenotypes of *abi4-1* were confirmed as described (Söderman et al., 2000, *Plant Physiol.* 124: 1752-1765) prior to use.

All seeds of the above-described lines were surface sterilized with 70% ethanol for 30 s and then with 6% household bleach for 5 min before being washed five times with sterile water. For aseptic growth, they were plated on solid medium containing Murashige and Skoog salts (Invitrogen), vitamins (Duchefa), 0.7% phytoagar (Invitrogen), and 1% sucrose and transferred to a tissue culture room. For soil growth, seedlings were transferred into individual pots 8-10 days after germination and maintained in the growth chamber. Plants were grown at 22°C under a 16-hr-light/8-hr-dark photoperiod aseptically or on soil.

The general development and growth phenotypes of the *attlp9-1* and *attlp9-2* knockout plants appear to be similar to those of the wild type plants. However, when seeds were plated on nutrient agar media, the germination time of mutant *attlp9-1* and *attlp9-2* seeds was advanced several hours compared with that of the wild type plants, whereas the selected sense line seeds (i.e., S13-2 and S16-1) germinated later than vector control seeds. It was found that 50% of the wild type seeds germinated after about 37 hour after plating. In contrast, 50% of the *attlp9-1* and *attlp9-2* knockout seeds germinated at hours 26-28 after plating, and 50% of the S13-2 and S16-1 seeds germinated around hours 40-42 hours after plating.

### Effect of ABA on Seed Germination of AtTLP9 Mutants and Overexpression Lines

It is known that seed germination is the outcome of an integration of many signals coordinated by the interactions of stage-specific developmental regulators and the competing effects of hormonal signals (Finkelstein et al., 2002, Curr. Opin. Plant. Biol. 5: 26-32). The most critical hormone promoting embryo maturation and preventing germination is ABA.

To determine whether the transgenic plants display altered ABA responses, the above-described lines were germinated on media containing various concentrations of ABA. Seeds collected at the same or similar times were used. After surface-sterilization, sterile seeds were suspended in 0.15% agarose, and kept in the dark at 4°C for 3 days to break residual dormancy. The seeds were then plated on agar plates in six replicates containing no ABA or 0.25, 0.5, 0.75, or 1.0µM ABA in 12-cm plastic petri dishes. Each agar plate was divided into seven sections, and 50 seeds of WT and AtTLP9 transgenic seeds were plated on each section. A seed was regarded as germinated when the radicle protruded through the seed coat.

In the presence of 1µM ABA, the germination of sense lines seeds was further delayed and the germination rate was reduced to less than 10%. In contrast, the germination rate of attlp9-1 and attlp9-2 mutant seeds nearly reached 50%, and about 30% of wild-type seeds were able to germinate in the presence of 1µM ABA. These results suggest that the disruption of the AtTLP9 gene affects the sensitivity of seeds to exogenous ABA.

In addition to reducing seed germination rate, ABA also inhibited the growth and the greening process in cotyledons of the sense transgenic lines. In MS agar medium containing 1µM ABA and 1 % sucrose, 90% of the 10-d-old seedlings showed developmental arrest although the radicles of most sense lines seeds emerged. In contrast, under the same conditions, attlp9-1 and attlp9-2 plants continued to grow and about 45% of the seedlings continued to develop true leaves, although at slower rates than abi4-1 mutant does. These results indicate that the alteration of AtTLP9 modulate plant's sensitivity to ABA during seed germination and early seedling development.

### AtTLP9 Expression is Transiently Up-regulated During Imbibition of Seeds

Real-time PCR experiments were conducted to quantify AtTLP9 transcript levels at seed maturation, seed germination, and early development stage. UBQ10 was used as the endogenous

control (Norris et al., 1993, Plant. Mol. Biol. 21: 895-906). Primers were designed using Primer Express 1.0 software (Applied Biosystems). The primers used were:

AtTLP9 forward primer, 5'-TAGGCCACACCGTGTAGTTCA-3';

AtTLP9 reverse primer, 5'-CGTCAACAGTCTCAACCCTAATCA-3';

5 UBQ10 forward primer, 5'- AGAAGTTCAATGTTTCGTTTCATGTAA -3'; and

UBQ10 reverse primer, 5'- GAACGGAAACATAGTAGAACACTTATTCA -3'.

The real-time PCR was performed in a 50  $\mu$ L reaction mixture containing 500 ng first strand cDNA, 2.5  $\mu$ M each primers and 1 $\times$ SYBR Green PCR Master Mix (Applied Biosystems). PCR cycling was 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 15 sec at 95°C/1  
10 min at 60°C. The UBQ10 mRNA quantity was set at '1' and AtTLP9 expression was determined relative to control samples. Threshold cycles were determined by Sequence Detection System V. 1.7a software (Applied Biosystems). The products were quantified by the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Scoresby, Victoria, Australia).

15 Seed germination is divided into three phases: imbibition, increased metabolic activity, and initiation of growth (Bewley. 1997, Plant Cell 9: 1055-1066). It was found that during seed maturation and seed imbibition at 4°C for 72h, AtTLP9 transcripts remained at a relatively low level. When the seeds were transferred to 22°C for further incubation, the levels rose after 8 h, peaked at 16h, and fell rapidly after 24h when the radicle emerged. The AtTLP9 transcripts were  
20 barely detectable afterwards. The transient expression of AtTLP9 indicated that AtTLP9 functions at stage II of seed germination as a checkpoint before radicle protrusion.

## OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the  
25 same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and  
30 conditions. Thus, other embodiments are also within the scope of the following claims.